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Spatial Distribution of the Emerging Foodborne Pathogen *Arcobacter* in the Gastrointestinal Tract of Pigs

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Abstract

Pigs are important reservoirs for *Arcobacter*. Since 1978, *Arcobacter* species have been associated with reproduction disorders, but excretion by clinically healthy pigs has been frequently reported as well. Information on *Arcobacter* colonization of the porcine gastrointestinal tract is lacking. In the present study, gastrointestinal tracts of 12 pigs were collected, and the content and mucus of eight sections were examined. Arcobacters were enumerated and isolated by a selective quantitative and qualitative method, respectively, and identified by multiplex–polymerase chain reaction (PCR). Their genetic diversity was examined by enterobacterial repetitive intergenic consensus PCR and pulsed-field gel electrophoresis. *Arcobacter* species were isolated from at least two gastrointestinal sections of all pigs in levels up to 10^5 colony-forming units (CFU) g^{-1} in content and 10^4 CFU g^{-1} in mucus. Characterization of the isolates revealed a high degree of genotypic diversity. In general, the highest counts, and greatest species and strain diversity was obtained from the large intestine, and especially from the rectum. Though *Arcobacter* strains were mostly detected in one gastrointestinal section, several unique strains were also recovered from the content and/or mucus of various gastrointestinal sections of individual pigs. In the gastrointestinal tract, *Arcobacter* is present with species distributions, numbers, and strain heterogeneity comparable to those reported on porcine carcasses post slaughter, thus confirming the potential route of transmission to carcasses by fecal contamination during processing.

Introduction

Since the creation in 1991 of the genus *Arcobacter* as a second genus within the family *Campylobacteraceae*, 13 species have been characterized (De Smet *et al*., 2011a; Figueras *et al*., 2011b; Houf *et al*., 2009; Vandamme *et al*., 1991). At present, six species are associated with humans and animals, whereas the others seem to be more environmentally related (Collado *et al*., 2011; Figueras *et al*., 2011a; Houf *et al*., 2009).

In humans, predominantly *Arcobacter butzleri* has been isolated from patients with enteritis and occasionally septicemia (Houf and Stephan, 2007). Infection probably occurs through the consumption of contaminated drinking water and food, in particular, poultry products, pork, and beef (De Smet *et al*., 2010; Ho *et al*., 2006a; Jacob *et al*., 1993; Van Driessche and Houf, 2007a). Other risk factors are contact with pets and person-to-person contact (Fera *et al*., 2009; Houf *et al*., 2008; Vandamme *et al*., 1992a).

Though the first reports of *Arcobacter* in farm animals described diseases such as reproduction disorders, mastitis, and enteritis (Ellis *et al*., 1978; Neill *et al*., 1982; Vandamme *et al*., 1992b), *Arcobacter* has by now been isolated from the feces of healthy farm animals all over the world (Van Driessche *et al*., 2003; Wesley *et al*., 2000).

Previous research has shown that pigs are an important *Arcobacter* reservoir (Ho *et al*., 2006b; Van Driessche *et al*., 2004), in contrast to broilers, from whose intestinal content *Arcobacter* species have rarely been recovered (Ho *et al*., 2008; Van Driessche and Houf, 2007b). Transfer of fecal material onto carcasses during slaughter processing is accepted as the major source for porcine carcass contamination (Van Driessche and Houf, 2007b). As with other foodborne pathogens, the gastrointestinal tract of the animals is also considered a preferential site for colonization and eventually excretion for *Arcobacter*. However, no information is currently available on the *Arcobacter* presence, or species and strain distribution within the porcine gastrointestinal tract. Therefore, the present study aims to determine the numbers, and species and strain diversity of *Arcobacter* in the different sections of the gastrointestinal tract of pigs at slaughter age.

Materials and Methods

Sampling of gastrointestinal content and mucus

In this study, gastrointestinal tracts of a total of 12 unrelated pigs (hereiafter identified as pigs 1–12, with mean weight of approximately 110 kg) were collected immediately

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post-mortem veterinary inspection on four occasions between March and October 2011 from two Belgian slaughterhouses. After both the esophagus and the terminal end of the rectum were tied off with plastic clips, the gastrointestinal tracts were individually packed in plastic bags, transported under cooled conditions, and processed within 2 h. In the laboratory, the stomach, and different sections of the small intestine (duodenum, jejunum, and ileum) and large intestine (caecum, colon ascendens, colon descendens, and rectum) were tied off and then cut out. The surface contamination was eliminated by immersing the sections in ethanol for 10 s, as previously described in Van Driessche and Houf (2007b). After evaporation of the ethanol in air, each section was opened with sterile utensils and a minimum of 1 g of the content was 1/10 diluted in *Arcobacter*-selective enrichment broth (Van Driessche *et al*., 2003). The broths were homogenized in a stomacher blender (IUL Instruments, Barcelona, Spain) (Van Driessche *et al*., 2003). In addition to the gastrointestinal content, mucus from each gastrointestinal section was collected from pigs 11 and 12. For this, the gastrointestinal sections were opened, and the content was sampled and then removed. To remove the remaining content, the sections were gently rinsed with phosphate-buffered saline (PBS), and the mucus was then scraped from the mucosa with a scalpel. The mucus samples were further processed as described above for the content samples.

Enumeration and isolation of Arcobacter

To determine the number of arcobacters within each section, 100 μL of each homogenate was directly inoculated in duplicate onto an *Arcobacter*-selective agar plate by spiral plating (Eddy Jet; IUL Instruments, Barcelona, Spain), as described previously by Van Driessche *et al*. (2003). The agar plates were incubated under microaerobic conditions for 48 h at 28°C in a jar from which 80% of the normal atmosphere had been evacuated and a gas mixture of 8% CO_2, 8% H_2, and 84% N_2 introduced. The plates were examined with a stereomicroscope with Henry transillumination, and the typically bluish colonies were counted (Houf and Stephan, 2007). A maximum of 30 colonies per gastrointestinal section per pig were randomly picked, subcultured onto blood agar plates, and incubated as described above.

To detect levels of *Arcobacter* below 10^2 colony-forming units (CFU) g^{-1}, a selective enrichment was performed by incubating all broths at 28°C for 48 h. Then, 100 μL of each broth was streaked onto an *Arcobacter*-selective agar plate and incubated as described above. Bacterial growth was checked every 24 h up to 72 h. One typical colony per agar plate was subcultured onto a blood agar plate.

The bacterial growth from the blood agar plates was stored in cryovials (Microbank; Pro-Lab Diagnostics, Richmond Hill, ON, Canada) at −80°C for later species identification and characterization.

Identification and characterization at strain level

Each isolate was cultivated again on a blood agar plate, and a 0.5-mL cell suspension was diluted in PBS to prepare the template DNA. Genomic DNA was extracted by the guanidium thiocyanate method (Pitcher *et al*., 1989). The DNA concentration was determined spectrophotometrically (BioPhotometer; Eppendorf, Hamburg, Germany) at 260 nm and adjusted to a concentration of about 50 ng μL^{-1}. Two microliters were used as a DNA template in the *Arcobacter* species–specific multiplex–polymerase chain reaction (PCR) assay of Douidah *et al*. (2010). Amplified products were size separated by agarose gel electrophoresis in 1% agarose Tris-Borate-EDTA gels at 100 V for 90 min.

A maximum of 12 colonies per *Arcobacter* species per gastrointestinal section per pig were randomly selected, and strains were characterized by enterobacterial repetitive intergenic consensus (ERIC)–PCR (Houf *et al*., 2002). The resulting banding patterns were used to evaluate the strain diversity comprising DNA fragments between 100 and 2072 bp. Computer-based normalization and interpolation of the DNA profiles and numerical analysis, using the Pearson product moment correlation coefficient with 1% position tolerance, were performed using the GelCompar 4.2 software package (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were constructed using the unweighted pair group linkage analysis method (UPGMA). For convenience, the correlation level was expressed as percentage similarity. As in previous studies, DNA patterns that differed by one or more DNA fragments were considered to be different genotypes (Aydin *et al*., 2007; Houf *et al*., 2003).

After ERIC-PCR analysis, genotypes that differed a maximum of two fragments and presented in more than one gastrointestinal section were subsequently characterized by pulsed-field gel electrophoresis (PFGE). The *Arcobacter* protocol by Son *et al*. (2006) was applied with *Kpn*I (Fermentas GmbH, St. Leon-Roth, Germany) as the restriction enzyme. The *Salmonella* Braenderup strain H9812, restricted with *Xba*I (Invitrogen, Paisley, UK), was used as molecular size standard. The PFGE patterns were analyzed using the GelCompar 4.2 software program, and clusters were constructed using the Dice coefficient and UPGMA. Patterns that differed in two or more fragments were considered to be different strains (Oporto *et al*., 2007). In addition, isolates showing similar *Kpn*I-PFGE patterns were further analyzed in a second PFGE with the restriction enzyme *Nru*I (Fermentas GmbH, Sankt Leon-Rot, Germany) using the PulseNet (*Sma*I) conditions for *Campylobacter jejuni* (Ribot *et al*., 2001).

Results

All pigs were clinically healthy according to *ante*- and *post-mortem* inspections by veterinarians, and content was present in each section of their gastrointestinal tract. *Arcobacter* spp. were isolated from two or more sections per pig. Numbers and species distribution are presented in Table 1. *Arcobacter* was isolated from the content of all sections examined, ranging from five pigs (duodenal and jejunal content) to 11 of the 12 (rectal content; Table 1).

In general, the highest *Arcobacter* numbers were present in the large intestine (except for pig 11). Except for two pigs (pigs 4 and 8), the highest *Arcobacter* numbers were counted in the rectal content. Ten pigs had, in certain sections, more than 100 *Arcobacter* colonies per gram, whereas in pigs 1 and 5, *Arcobacter* was only detected after selective enrichment of the samples. The *Arcobacter* counts in the different gastrointestinal sections of the 12 pigs are also presented in Table 1. *Arcobacter* species were not always isolated from the mucus of one gastrointestinal section, although they were isolated from the corresponding content, and vice versa.

Table 1. *Arcobacter* Load and Species Distribution in the Different Sections of the Gastrointestinal Tract and in Mucus

	Gastrointestinal sections							
Pig	*Stomach*	*Duodenum*	*Jejunum*	*Ileum*	*Caecum*	*Colon ascendens*	*Colon descendens*	*Rectum*
1	—	—	Ac[a]	—	Ac	—	—	—
2	—	—	—	—	—	—	Ac	Ac, At (4.3)
3	Ab	Ab	—	—	Ab (1.9)	Ab (2.2)	Ab (1.9)	Ab, Ac (3.5)
4	—	—	—	Ab (1.9)	Ab, Ac, As (4.2)	Ab, Ac (3.1)	Ac (1.9)	Ac (3.6)
5	—	—	—	—	—	Ab	—	Ac
6	—	Ac	Ab, Ac (2.3)	Ac (3.1)	Ac	—	—	Ab, Ac, At (5.1)
7	Ac (1.9)	—	—	Ac	—	—	—	Ac, Aci (4.7)
8	Ac (3.0)	Ac (2.0)	—	Ac (1.9)	Ac (1.9)	Ac	Ac (5.2)	Ab, Ac, Aci (5.1)
9	Ac	—	—	Ac	Ac (1.9)	—	Aci	Ab, Ac (3.2)
10	—	—	Ac	Ac	Ac	At	Ab	Ac, Aci, At (5.0)
11	Ab, Ac (2.4)	Ab, Ac (2.9)	Ab, Ac (4.7)	Ab	Ab, Ac (2.7)	Ab (3.4)	Ab (3.5)	Ab, Ac (3.9)
11-MUCUS	Ab, Ac (3.2)	—	Ab, Ac (4.4)	—	Ab, Ac (3.0)	Ab (2.2)	Ab (2.7)	Ab, Ac, Aci (2.8)
12	Ac	Ab	Ab	—	Ab	—	—	Ab, Ac, Aci (4.9)
12-MUCUS	—	—	Ab	—	Ab	—	—	Ab, Ac, Aci (3.0)

[a]If log values are not given, arcobacters were only isolated after enrichment ($<10^2$ colony-forming units [CFU] g^{-1}).

—, no isolation of arcobacters; Ab, *Arcobacter butzleri* ; Ac, *Arcobacter cryaerophilus*; Aci, *Arcobacter cibarius*; As, *Arcobacter skirrowii*; At, *Arcobacter thereius.*

( ): *Arcobacter* counts are presented in log N g^{-1} content or mucus so that $2.8=10^{2.8}$ and $4.7=10^{4.7}$.

Five animal associated species were present in the pigs (Table 1). The species *A. cibarius*, *A. thereius,* and *A. skirrowii* were only recovered after direct plating, but both *A. cryaerophilus* and *A. butzleri* were also isolated after enrichment. In general, only one *Arcobacter* species (either *A. butzleri* or *A. cryaerophilus*) was isolated from the content of the stomach and small intestine of the pigs. The highest *Arcobacter* species diversity was present in the large intestine and, except for pigs 4 and 5, in the rectum. *Arcobacter cryaerophilus* and *A. butzleri* were isolated at least once from all gastrointestinal sections of the pigs. *Arcobacter cibarius* was recovered from the rectal content of pigs 7, 8, 10, and 12, and the colon descendens of pig 9. Pigs 2, 6, and 10 had *A. thereius* in the rectal content, and *A. skirrowii* was only present in the caecal content of pig 4. One animal (pig 10) was infected with four *Arcobacter* species simultaneously (Table 1). Only one pig (1) carried a single *Arcobacter* species. Except for the rectal content of pig 11, the species isolated from the mucus were identical to those from the corresponding content.

In the present study, 384 *Arcobacter* isolates were characterized by ERIC-PCR, and 179 genotypes were distinguished. Genotypes with the same or similar banding patterns by ERIC-PCR analysis and present in more than one gastrointestinal section were also typed by PFGE. The *Arcobacter* strain distribution within each pig is shown in Table 2. In most cases, PFGE analysis confirmed the presence of 131 *A. cryaerophilus*, 30 *A. butzleri*, 14 *A. cibarius*, three *A. thereius,* and one *A. skirrowii* strains. Additional restriction analysis with *Nru*I always confirmed the results obtained with *Kpn*I. Figure 1 presents the banding patterns obtained with ERIC-PCR (Fig. 1A) of an identical *A. butzleri* genotype consisting of isolates from the content of the stomach, duodenum, caecum, colon ascendens, colon descendens, and rectum of pig 3. However, an additional fragment was observed for the caecal isolate (pig 3, caecum E) after PFGE analysis with both restriction enzymes (Fig. 1B,C). Another indistinguishable *A. cryaerophilus* genotype consisting of isolates from the stomach, duodenum and colon ascendens was obtained from pig 8, by ERIC-PCR. After PFGE with both restriction enzymes one duodenal isolate lacked a fragment possessed by the other strains (data not shown). In the present study, a certain *Arcobacter* strain was only present in the gastrointestinal tract of one pig. In many times, these strains were also recovered from a single specific section of the gastrointestinal tract only. Despite the fact that *Arcobacter* strains were mostly detected in one gastrointestinal region, several strains were recovered from the content of various gastrointestinal sections in eight pigs (Table 2, marked with an underscore). In total, four *A. butzleri* and nine *A. cryaerophilus* strains were isolated from the gastrointestinal content of up to six and three gastrointestinal sections, respectively (Table 2). In addition, from both the content and mucus of the same gastrointestinal sections of individual pigs, three *A. butzleri*, five *A. cryaerophilus,* and one *A. cibarius* strains were recovered. The greatest strain diversity was detected in the rectal content.

Discussion

Pigs are important reservoirs for *Arcobacter* species (Hume *et al*., 2001; Van Driessche *et al*., 2003), and their excretion in porcine feces has frequently been reported (De Smet *et al*., 2011b; Van Driessche *et al*., 2004). However, information on their spatial distribution in the porcine gastrointestinal tract has been lacking. In the present study, *Arcobacter* was isolated from at least two gastrointestinal sections of all 12 pigs in numbers up to 10^5 CFU g^{-1}. The absence of *Arcobacter* in certain sections can be explained either by the possibility that they really were absent or that their numbers were below the detection limit of the isolation protocol applied. Both *A. butzleri* and *A. cryaerophilus* were isolated from the content of all gastrointestinal sections, in contrast to *A. cibarius*, *A. thereius,* and *A. skirrowii*. Van Driessche *et al*. (2003) suggested that the

Table 2. Distribution of *Arcobacter* Strains in the Different Sections of the Gastrointestinal Tract

Pig	*Sections of the gastrointestinal tract*							
	Stomach	*Duodenum*	*Jejunum*	*Ileum*	*Caecum*	*Colon ascendens*	*Colon descendens*	*Rectum*
1	—	—	C-1	—	C-2	—	—	—
2	—	—	—	—	—	—	C-3	C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, T-1
3	B-1	B-1	—	—	B-1	B-1	B-1	B-1, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21
4	—	—	—	B-2	C-22, C-23, C-24, C-25, B-2, B-3, B-4, B-5, S-1	B-2, C-22, C-26	C-26, C-27	C-22, C-28, C-29, C-30, C-31, C-32, C-33, C-34, C-35
5	—	—	—	—	—	B-7	—	C-36
6	—	C-37	B-7, C-38	C-38, C-39	C-38	—	—	C-40, C-41, C-42, C-43, C-44, C-45, C-46, C-47, C-48, C-49, T-1, T-2
7	C-50	—	—	C-51	—	—	—	C-51, C-52, C-53, C-54, C-55, C-56, C-57, C-58, C-59, Ci-1, Ci-2
8	C-60, C-61, C-62, C-63, C-64, C-65	C-60, C-61, C-66	—	C-66, C-67	C-68	C-61	C-66, C-69, C-70, C-71, C-72, C-73	C-69, C-74, C-75, C-76, C-77, C-78, C-79, C-80, C-81, C-82, Ci-3, B-8
9	C-83	—	—	C-84	C-85, C-86	—	Ci-4	B-9, C-87, C-88, C-89, C-90, C-91
10	—	—	C-92	C-93	C-94	T-3	B-10	B-10, Ci-5, Ci-6, Ci-7, Ci-8, C-95, C-96, C-97, C-98, C-99, C-100
11	C-101, C-102, C-103, B-11, B-12, B-13	C-101, C-104, B-11, B-14, B-15	C-105, C-106, B-11, B-16, B-17	B-11	C-107, B-11, B-16	B-11, B-16	B-11	C-104, C-107, C-108, C-109, C-110, C-111, C-112, B-11, B-18, B-19, B-20
11-mucus	B-11, B-12, B-14, B-21, C-101, C-104, C-105	—	C-102, C-105, C-106, C-113, C-114, B-11, B-22	—	B-11, C-102, C-104, C-105, C-115, C-116	B-11	B-11	Ci-9, Ci-10, Ci-11, B-11, B-14, C-117, C-118
12	C-119	B-23	B-24	—	B-23	—	—	C-120, C-121, C-122, C-123, C-124, C-125, C-126, C-127, B-25, B-26, B-27, B-28, Ci-12, Ci-13, Ci-14
12-mucus	—	—	C-29	—	B-23	—	—	C-119, C-128, C-129, C-130, C-131, Ci-12, B-30

The same genotype numbers in the content and/or mucus (which are underlined) of different gastrointestinal sections of an individual pig, correspond to the presence of similar genotypes in these gastrointestinal sections.

—, no isolation of arcobacters; B, *A. butzleri*; C, *A. cryaerophilus*; Ci, *A. cibarius*; T, *A. thereius.*

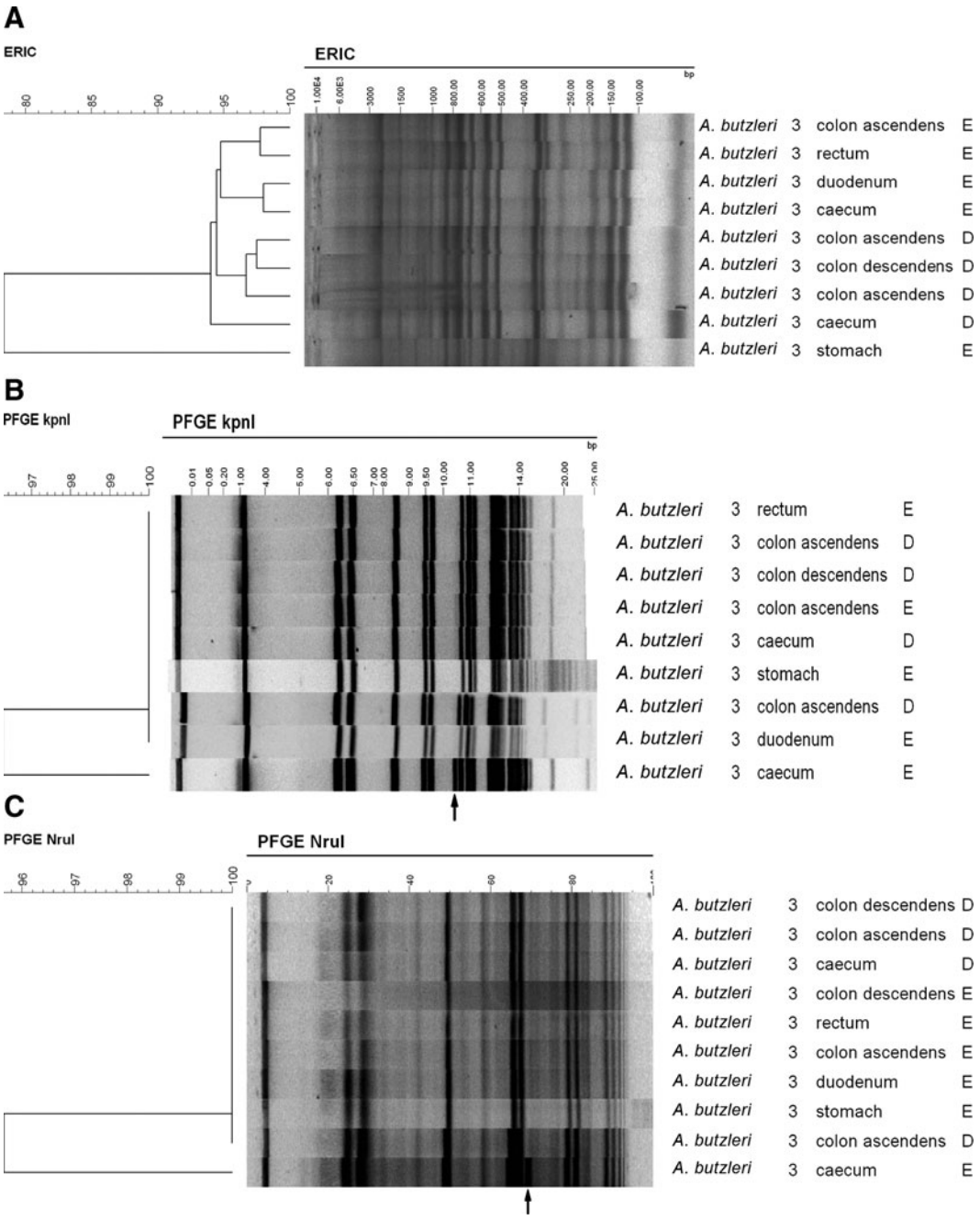

FIG. 1. Characterization of the *Arcobacter butzleri* isolates present in the content of six sections (stomach, duodenum, caecum, colon ascendens, colon descendens, and rectum) of the gastrointestinal tract of pig 3 by enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) **(A)** and pulsed-field gel electrophoresis (PFGE) [restriction enzymes *Kpn*I **(B)** and *Nru*I **(C)**]. An additional fragment (indicated by an arrow) was obtained for one of the isolates (three caecum E) by PFGE with the restriction enzymes *Kpn*I and *Nru*I. **(A)** The ERIC-PCR clusters were constructed using the Pearson product moment correlation coefficient with 1% position tolerance, and the unweighted pair group linkage analysis method (UPGMA). **(B,C)** The PFGE pattern clusters were constructed using the Dice coefficient and UPGMA. D, isolate obtained after direct plating; E, isolate obtained after enrichment.

isolation method used can bias the study outcome because certain *Arcobacter* species may be better adapted to the isolation media and procedure, especially when an enrichment step is applied. However, this possibility was excluded from this study as both a direct isolation and enrichment of a previously validated *Arcobacter* isolation method were applied (Houf *et al*., 2001; Vandamme *et al*., 1991; Van Driessche *et al*., 2003).

Some *A. butzleri* and *A. cryaerophilus* strains were isolated from every section of the gastrointestinal tract. In an *Arcobacter* infection study with 1-day-old piglets, *A. butzleri* was also the longest excreted species in the feces (up to 10 days post-infection), suggesting an intestinal colonization and multiplication, in contrast to *A. skirrowii* and *A. cryaerophilus*, which displayed a shorter duration of shedding (Wesley *et al*.,

1996). *Arcobacter butzleri* was also isolated from the internal organs of infected piglets, which suggested that *A. butzleri* can penetrate the intestinal barrier and represents a more invasive and virulent species (Wesley *et al*., 1996). Moreover, *A. butzleri* induced epithelial barrier dysfunctions by changes in tight junction proteins and induction of epithelial apoptosis in human HT-29/B6 colonic epithelial monolayers (Bücker *et al*., 2009). From pigs 11 and 12, *A. butzleri*, *A. cryaerophilus*, and *A. cibarius* were isolated from the gastrointestinal mucus. Genome sequence analysis of *A. butzleri* has revealed several common features with *Campylobacter*, such as putative virulence genes and the inability to utilize sugars as carbon sources (Miller *et al*., 2007). Mucus components such as mucin are major chemoattractants and can explain the presence of *Arcobacter* (Hugdahl *et al*., 1988).

Several *Campylobacter* studies in pigs and cattle have already demonstrated that different species appear to preferentially colonize different sections of the gastrointestinal tract (Inglis *et al*., 2005; Madden *et al*., 2007). This may be explained by the fact that microenvironmental conditions such as oxygen tension, pH, host receptors, and microflora vary substantially in these different sections (Inglis *et al*., 2005). Moreover, host species differences in mucus or associated factors can even transform the outcome of a *C. jejuni* infection from being a pathogen in humans to a commensal behavior in another host species. Byrne *et al*. (2007) demonstrated that, compared to human mucus, chicken-derived mucus attenuated *C. jejuni* virulence.

The high diversity of *Arcobacter* strains is once again demonstrated in the present study. In general, the highest numbers, species, and strain diversity were obtained from the rectum. As transfer of fecal material onto carcasses during the slaughter process is regarded as the main contamination source (Van Driessche and Houf, 2007b), their presence in the rectum is of primordial interest in respect to food safety.

One *A. butzleri* and eight *A. cryaerophilus* strains were recovered in levels up to 10^3 CFU g^{-1} of stomach content (Table 2). After their isolation from the stomach, one of the *A. butzleri* and two *A. cryaerophilus* strains were recovered from the content of five, one, and two intestinal sections, respectively. Suarez *et al*. (1997) earlier detected *A. butzleri* and *A. cryaerophilus* in 52.4% of the pig stomachs with evidence of gastric pathology, although they were also found in 50.8% of the normal stomachs. Their primary role in gastric ulcerative disease remains unclear and should be further investigated. In previous studies, direct plating revealed a larger diversity of *Arcobacter* strains than in enrichment, where only one genotype was recovered (De Smet *et al*., 2011b; Van Driessche *et al*., 2004). Although the majority of the *Arcobacter* strains were recovered from one gastrointestinal region only, several strains were recovered from the content of up to six gastrointestinal sections. As already mentioned for the species level, there is a possibility that different strains have adapted to colonize different sections of the gastrointestinal tract. For *C. jejuni*, differences in colonization types may be due to genetic differences or differences in gene expression of colonization/invasion-related genes (Hänel *et al*., 2009).

In conclusion, *Arcobacter* species are present in the gastrointestinal tract of pigs with a species distribution, number, and strain heterogeneity comparable to those reported on porcine carcasses post slaughter (96.4%) (De Smet *et al*., 2011b; Van Driessche and Houf, 2007b). This finding further implicates the potential route of transmission to carcasses by fecal contamination during processing. However, due to the great heterogeneity within an animal, typing *Arcobacter* isolates seems not to contribute to the identification of initial contamination sources. Furthermore, it could not be elucidated whether *Arcobacter* or some particular strains truly colonize the intestines of pigs or are just passengers of the gastrointestinal tract. These pathways should probably be taken into account, as evidence for both have been demonstrated. However, this means that pigs are constantly being infected by arcobacters present in their environment. For the latter, this could not be confirmed in a previous study on the epidemiology of *Arcobacter* during the fattening period (De Smet *et al*., 2011b).

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Disclosure Statement

No competing financial interests exist.

References

Aydin F, Gümüşsoy KS, Atabay HI, Ia T, Abay S. Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J Appl Microbiol 2007;103:27–35.

Bücker R, Troeger H, Kleer J, Fromm M, Schulzke JD. *Arcobacter butzleri* induced barrier dysfunction in intestinal HT-29/B6 cells. J Infect Dis 2009;200:756–764.

Byrne CM, Clyne M, Bourke B. *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. Microbiology 2007;153:561–569.

Collado L, Levican A, Perez J, Figueras MJ. *Arcobacter defluvii* sp. nov., isolated from sewage samples. Int J Syst Evol Microbiol 2011;61:2155–2161.

De Smet S, De Zutter L, Van Hende J, Houf K. *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. J Appl Microbiol 2010;108:299–305.

De Smet S, Vandamme P, De Zutter L, On SLW, Douidah L, Houf K. *Arcobacter trophiarum* sp. nov. isolated from fattening pigs. Int J Syst Evol Microbiol 2011a;61:356–361.

De Smet S, De Zutter L, Debruyne L, Vangroenweghe F, Vandamme P, Houf K. *Arcobacter* population dynamics in pigs on farrow-to-finish farms. Appl Environ Microbiol 2011b;77:1732–1738.

Douidah L, De Zutter L, Vandamme P, Houf K. Identification of five human and mammal associated *Arcobacter* species by a novel multiplex-PCR assay. J Microbiol Methods 2010;80:281–286.

Ellis WA, Neill SD, O'Brien JJ, Hanna J. Isolation of *Spirillum*-like organisms from pig fetuses. Vet Rec 1978;102:106.

Figueras MJ, Collado L, Levican A, Perez J, Solsona MJ, Yustes C. *Arcobacter molluscorum* sp. nov., a new species isolated from shellfish. Syst Appl Microbiol 2011a;34:105–109.

Figueras MJ, Levican A, Collado L, Inza MI, Yustes C. *Arcobacter ellisii* sp. nov., isolated from mussels. Syst Appl Microbiol 2011b;34:414–418.

Hänel I, Borrmann E, Müller J, Müller W, Pauly B, Liebler-Tenorio EM, Schulze F. Genomic and phenotypic changes of *Campylobacter jejuni* strains after passage of the chicken gut. Vet Microbiol 2009;136:121–129.

Ho HTK, Lipman LJA, Gaastra W. *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent! Vet Microbiol 2006a;115:1–13.

Ho TKH, Lipman LJA, van der Graaf-van Bloois L, van Bergen M, Gaastra W. Potential routes of acquisition of *Arcobacter* species by piglets. Vet Microbiol 2006b;114:123–133.

Ho HTK, Lipman LJA, Gaastra W. The introduction of *Arcobacter* spp. in poultry slaughterhouses. Int J Food Microbiol 2008; 125:223–229.

Houf K, Stephan R. Isolation and characterization of the emerging foodborne pathogen *Arcobacter* from human stool. J Microbiol Methods 2007;68:408–413.

Houf K, Devriese LA, De Zutter L, Van Hoof J, Vandamme P. Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products. Int J Food Microbiol 2001;71:189–196.

Houf K, De Zutter L, Van Hoof J, Vandamme P. Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. Appl Environ Microbiol 2002;68:2172–2178.

Houf K, De Zutter L, Verbeke B, Van Hoof J, Vandamme P. Molecular characterization of *Arcobacter* isolates collected in a poultry slaughterhouse. J Food Prot 2003;66:364–369.

Houf K, De Smet S, Baré J, Daminet S. Dogs as carriers of the emerging pathogen *Arcobacter*. Vet Microbiol 2008;130: 208–213.

Houf K, On SLW, Coenye T, Debruyne L, De Smet S, Vandamme P. *Arcobacter thereius* sp. nov., isolated from pigs and ducks. Int J Syst Evol Microbiol 2009;59:2599–2604.

Hugdahl MB, Beery JT, Doyle MP. Chemotactic behavior of *Campylobacter jejuni*. Infect Immun 1988;56:1560–1566.

Hume ME, Harvey RB, Stanker LH, Droleskey RE, Poole TL, Zhang HB. Genotypic variation among *Arcobacter* isolates from a farrow-to-finish swine facility. J Food Prot 2001;64:645–651.

Inglis GD, Kalischuk LD, Busz HW, Kastelic JP. Colonization of cattle intestines by *Campylobacter jejuni* and *Campylobacter lanienae*. Appl Environ Microbiol 2005;71:5145–5153.

Jacob J, Lior H, Feuerpfeil I. Isolation of *Arcobacter butzleri* from a drinking water reservoir in Eastern Germany. Zbl Hyg 1993;193:557–562.

Madden RH, Moran L, Scates P. Diversity of *Campylobacter coli* genotypes in the lower porcine gastrointestinal tract at time of slaughter. Lett Appl Microbiol 2007;45:575–580.

Miller WG, Parker CT, Rubenfield M, Mendz GL, Wösten MMSM, Ussery DW, Stolz JF, Binnewies TT, Hallin PF, Wang G, Malek JA, Rogosin A, Stanker LH, Mandrell RE. The complete genome sequence and analysis of the Epsilonproteobacterium *Arcobacter butzleri*. PLos ONE 2007;2:1–21.

Neill SD, Mackie DP, Logan EF. *Campylobacter* mastitis in dairycows. Vet Rec 1982;110:505–506.

Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A. Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swine farms. J Appl Microbiol 2007;103:977–984.

Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 1989;8:151–156.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol 2001;39:1889–1894.

Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Genetic diversity of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. J Food Prot 2006;69:1028–1033.

Suarez DL, Wesley IV, Larson DJ. Detection of *Arcobacter* species in gastric samples from swine. Vet Microbiol 1997;57:325–336.

Van Driessche E, Houf K, Van Hoof J, De Zutter L, Vandamme P. Isolation of *Arcobacter* species from animal feces. FEMS Microbiol Lett 2003;229:243–248.

Van Driessche E, Houf K, Vangroenweghe F, Nollet N, De Zutter L, Vandamme P, Van Hoof J. Occurence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. Res Microbiol 2004;155:662–666.

Van Driessche E, Houf K. Discrepancy between the occurrence of *Arcobacter* in chickens and broiler carcass contamination. Poultry Sci 2007a;86:744–751.

Van Driessche E, Houf K. Characterization of the *Arcobacter* contamination in Belgian pork carcasses and raw retail pork. Int J Food Microbiol 2007b;118:20–26.

Vandamme P, Falsen E, Rossau R, Hoste B, Segers P, Tytgat R, De Ley J. Revision of *Campylobacter*, *Helicobacter* and *Wolinella* taxonomy: Emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int J Syst Bacteriol 1991;41:88–103.

Vandamme P, Vancanneyt M, Pot B, Mels L, Hoste B, Dewettinck D, Vlaes L, Van Den Borre C, Higgins R, Hommez J, Kesters K, Butzler JP, Goossens H. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov, an aerotolerant bacterium isolated from veterinary specimens. Int J Syst Bacteriol 1992a;42:344–356.

Vandamme P, Pugina P, Benzi G, Vanetterijck R, Vlaes L, Kersters K, Butzler JP, Lior H, Lauwers, S. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. J Clin Microbiol 1992b;30:2335–2337.

Vandenberg O, Dediste A, Houf K, Ibekwem S, Souayah H, Cadranel S, Douat N, Zissis G, Butzler JP, Vandamme P. *Arcobacter* species in humans. Emerg Infect Dis 2004;10: 1863–1867.

Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991;19:6823–6831.

Wesley IV, Baetz AL, Larson DJ. Infection of cesarean-derived colostrums-deprived 1-day-old piglets with *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. Infect Immun 1996;64:2295–2299.

Wesley IV, Wells SJ, Harmon KM, Green A, Schroeder-Tucker L, Glover M, Siddique I. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. Appl Environ Microbiol 2000; 66:1994–2000.

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